

What is claimed is:

1. A genetically engineered vector comprising:

a) a 5' gene trap cassette, comprising in operable
5 combination:

- 1) a splice acceptor;
- 2) a first exon sequence located 3' to said splice
acceptor, said first exon encoding a marker
enabling the identification of a cell expressing
10 said exon; and
- 3) a polyadenylation sequence defining the 3' end
of said first exon;

b) a 3' gene trap cassette located 3' to said
polyadenylation sequence, comprising in operable
15 combination:

- 1) a promoter;
- 2) a second exon sequence located 3' from and
expressed by said promoter, said second exon being
synthetically derived and not encoding an activity
20 conferring antibiotic resistance;
- 3) a splice donor sequence defining the 3' region
of the exon; and

wherein said vector does not encode a promoter mediating the
expression of said first exon, and wherein said vector does
25 not encode a sequence that mediates the polyadenylation of an
mRNA transcript encoded by said second exon sequence and
expressed by said promoter.

2. The vector according to Claim 1 wherein said first exon
30 additionally encodes an internal ribosome entry site
operatively positioned between said splice acceptor and the
initiation codon of said first exon.

3. The vector of Claim 1 wherein said second exon and splice donor sequences are derived from a naturally occurring eukaryotic gene.

5 4. The vector of Claim 1 additionally incorporating a mutagenic mini-exon sequence operatively positioned upstream from said splice acceptor site.

10 5. The vector of Claim 1 additionally incorporating in the region between said polyadenylation sequence and said promoter at least one mutagenesis enhancer drawn from the group consisting of a transcription termination sequence, a 3' terminal exon, a sequence encoding a self-cleaving RNA, and an exon that changes the reading frame.

15 6. The vector of Claim 1 wherein said first exon encodes a marker drawn from the group consisting of a marker conferring antibiotic resistance, a marker conferring antibiotic sensitivity, an enzymatic marker, a recombinase and a
20 fluorescently detectable marker.

7. The vector of claim 6 wherein said marker encodes neomycin resistance.

25 8. A genetically engineered retroviral vector comprising:
a) a marker gene expressed by a first vector encoded promoter; and
b) a 3' gene trap cassette, comprising in operable combination:

30 1) a second vector encoded promoter;
2) an exon sequence located 3' from and expressed by said second promoter, said exon not encoding an activity conferring antibiotic resistance;
3) a splice donor sequence defining the 3' region
35 of the exon; and

wherein said vector does not encode a sequence that mediates the polyadenylation of an mRNA transcript encoded by said exon sequence.

5 9. An infectious retrovirus having a genome produced by a vector according to one of Claims 1 or 8.

10. The use of a retrovirus according to Claim 9 to trap a gene in a eukaryotic target cell or organism.

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11. The use of a vector according to Claims 1 or 8 to trap a gene in a eukaryotic target cell wherein said vector is introduced into said target cell by a method drawn from the group consisting of electroporation, viral infection, retrotransposition, microinjection and transfection.

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12. A transgenic cell incorporating a vector according to any one of Claims 1 or 8 into the genome of the cell.

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13. A transgenic non-human animal that has been genetically modified to incorporate a vector according to any one of Claims 1 or 8 into the genome of one or more cells in the animal.

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14. The use of a vector according to any one of Claims 1 or 8 to activate the expression of a naturally occurring gene in a cell.

15. The use of claim 14 wherein said cell is mammalian.

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16. The use of claim 15 wherein said mammalian cell is a human cell.

17. The use of a 3' gene trap cassette to alter the expression of a cellularly encoded gene, said 3' gene trap cassette comprising in operable combination:

- 1) a promoter;
- 2) an exon sequence located 3' from and expressed by said promoter, said exon not encoding an activity conferring antibiotic resistance; and
- 3) a splice donor sequence defining the 3' region of said exon

wherein said cassette is non-homologously incorporated into the genome of a eukaryotic target cell and said splice donor sequence of the transcript encoded by said exon is spliced to a splice acceptor sequence of said cellularly encoded gene.

18. The use of Claim 17 wherein said non-homologously incorporated cassette is present in a retroviral vector that has nonspecifically integrated into the genome of the eukaryotic target cell.

19. The use of Claim 18 wherein said exon is not encoded by the target cell genome or not normally expressed by the target cell genome.

20. A process for obtaining novel eucaryotic polynucleotide sequence information comprising:

a) introducing into a eucaryotic cell a 3' gene trap cassette, comprising in operable combination:

- 1) a promoter;
- 2) an exon sequence located 3' from and expressed by said promoter, said exon not encoding an activity conferring antibiotic resistance;
- 3) a splice donor sequence defining the 3' region of the exon;

b) maintaining the cell under conditions allowing the nonspecific or nontargeted integration of the gene trap cassette into the genome of the cell;

5 c) obtaining the chimeric transcript resulting from the splicing of said exon from said 3' gene trap cassette to a second exon encoded by the genome of said eucaryotic cell;

d) reverse transcribing said chimeric transcript in vitro to produce a cDNA template; and

10 e) determining the polynucleotide sequence of the cDNA from step d.